PRODUCTION OF EYE COLOR HORMONE BY THE EYES OF DROSOPHILA MELANOGASTER [1]

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Introduction

Two diffusible substances, known as v^+ and cn^+ hormones, are involved in the differentiation of certain eye colors in *Drosophila melanogaster* (Ephrussi, 1938). Evidence that eye tissues produce the hormones was presented by Beadle and Ephrussi (1936) and by Ephrussi and Chevais (1938).

The experiments described here were designed: (1) to determine the time at which v^+ hormone appears in the optic anlagen of wild type and brown flies; (2) to measure the amount of hormone present in the optic discs at various stages of development after its appearance there; and (3) to test directly, by means of transplantation, the production of v^+ hormone by wild type optic anlagen.

Materials and Methods

The techniques used for collection of pupae, transplantation, and injection of extracts are essentially those described by Ephrussi and Beadle (1936). Ages of larvae were measured from egg laying (eggs collected over a four-hour period), those of pupae from puparium formation, and those of adults from emergence. All cultures were kept at 25° C. Wild type flies used were F_1 offspring of a cross of the wild type stocks Canton-S by Oregon-R.

Extracts of optic discs were prepared in the following manner: After dissection from the animals, optic anlagen were transferred to 0.01 cc. of Ringer's solution contained in a small glass tube. The latter was heated (after the addition of each 5 pairs of discs) by partially immersing it in boiling water in order to destroy hormone-inactivating enzymes (Thimann and Beadle, 1937). The extracted discs were then separated from the fluid by centrifuging the fluid through a sintered glass filter fused into the bore of a small glass tube, the lower closed

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portion of which received the extract. The elongate receiving tube could then be separated from the filter, and, when desired, the contained extract could be hermetically sealed, sterilized, and stored. In most cases, however, extracts were injected at once. In all cases 0.01 cc. of Ringer's solution was used, and in most cases 25 pairs of optic discs extracted.

Extracts were tested for quantity of hormone by injecting 1.4 mm.^{3} (the maximum volume practicable) into mature larvae of the standard test animal, vermilion brown, and the eye color of the emerged flies compared with the series of genetic standards developed and described by Tatum and Beadle (1938).[2] The color modification of the eyes of injected test animals as measured by the scale of Tatum and Beadle (1938) varies logarithmically with the amount of hormone injected. Hence, color values can be converted directly into arbitrary units of hormone. The amounts of hormone contributed per donor fly were then calculated and are listed in Tables I, II, and III as "units per donor fly."

Optic disc extracts were made from: (1) wild type larvae approximately 120 hours old; (2) brown and wild type pupae of various ages; and (3) brown and wild type adults of various ages. In the transplant experiments, wild type optic discs from larvae close to puparium formation were transplanted into vermilion brown larvae of the same age. Transplants were recovered from the hosts on emergence, extracts prepared and tested as indicated above.

Experimental

Extracts of Larval, Pupal, and Adult Eyes

From tests of three extracts, one made from 40 pairs and two from 100 pairs of larval discs, no eye color modification showed in the 12 test flies recovered. Hence it can be concluded that the hormone is not present in the eyes in detectable amounts before puparium formation.

Table I summarizes the data obtained from extracts of pupal eyes at various stages of development. In both brown and wild type, the hormone first occurs in the anlagen between 35 and 40 hours after puparium formation. The maximum color modification for each extract (shown in Fig. 1) is plotted against the age of donors after puparium formation. It is seen that the two series are similar; the amount of hormone increases rapidly during the first 15 to 20 hours after its appearance,

[2] No standards were available for values between 2.0 and 3.5; thus classifications from about 2.4 to 3.0 may not be accurate.

reaching, in the case of wild type, a maximum at 3.5, which is maintained until emergence. In the brown series this value of 3.5 was obtained twice, i.e. for 80-hour pupae and for emerging flies; other maximal values obtained during pupal development range from 2.8 to 3.0. However, because the differences between the high values of brown and of wild type are no greater than the variations in the wild

TABLE I

Production of v^+ hormone by the eyes of wild type and brown pupae.

Donors		No. of Hosts		Color Values		Units per donor*
Age in hours	Number	Injected	Emerged	Range	Mean	
Wild type						
31	25	6	4	0.0	0.00	0.00
31	25	6	5	0.0	0.00	0.00
36	50	5	4	0.0	0.00	0.00
36	90	9	6	0.0	0.00	0.00
42	32	5	3	0.2–0.4	0.30	0.14
48	25	7	6	1.1–1.5	1.10	0.47
50	25	8	7	0.8–1.6	1.14	0.48
50	25	5	2	2.2–2.4	2.30	1.03
51	23	6	6	0.6–2.0	1.17	0.79
55	25	6	6	1.2–2.0	1.70	0.72
55	25	6	3	0.7–2.0	1.57	0.72‡
60	25	7	5	2.3–3.5	3.03	2.66
60	25	7	7	1.0–2.2	1.86	0.88
60	25	6	4	1.2–1.8	1.57	0.61‡
65	25	8	6	1.8–2.2	2.07	0.88
65	25	5	4	1.5–2.0	1.87	0.72‡
70	25	9	8	0.8–3.5	2.18	2.66
70	25	6	2	2.0–3.0	2.50	1.70†
70	25	6	4	2.0	2.00	0.72‡
75	25	9	8	1.2–3.5	1.98	2.66
75	25	7	1	2.8	2.80	1.44†
80	25	8	7	1.5–3.0	2.14	1.70
90	24	6	1	3.5	3.50	2.74†
91	25	7	7	3.0–3.5	3.36	2.66
95	25	9	3	2.0–3.5	3.00	2.66

TABLE I (*Continued*)

Donors		No. of Hosts		Color Values		Units per donor*
Age in hours	Number	Injected	Emerged	Range	Mean	
Brown						
35	25	7	6	0.0	0.00	0.00
40	25	6	5	0.0–0.2	0.10	0.16
45	25	5	2	0.5	0.50	0.19
50	25	6	4	1.5–2.0	1.78	0.72‡
51	25	7	6	0.8–2.1	1.33	0.84
55	25	5	5	2.0–2.8	2.36	1.44
55	25	9	8	1.5–3.0	1.98	1.70
61	25	6	5	1.1–2.4	1.98	1.03
61	25	5	1	1.7	1.70	0.51†
65	25	7	6	1.5–2.8	2.13	1.44
65	25	10	8	0.8–2.0	1.83	0.72§
70	25	6	6	2.1–2.8	2.52	1.44
70	25	6	3	0.8–2.0	1.53	0.72‡
70	25	4	1	1.5	1.50	0.47†
70	25	6	1	1.2	1.20	0.37†
75	25	8	5	2.1–2.8	2.44	1.44
75	25	5	3	2.3–2.5	2.40	1.13
75	25	7	1	2.0	2.00	0.72†
80	25	6	4	2.5	2.50	1.13‡
80	25	6	4	0.8–3.5	2.83	2.66
85	25	6	2	2.2–3.0	2.60	1.70‡
90	25	7	5	1.0–2.0	1.60	0.72‖
95	25	7	4	2.5–3.2	2.85	2.03
95	25	6	4	1.5–3.0	2.50	1.70

* Values calculated from the maximal color modifications.

† Not reliable statistically.

‡ Some hormone may have been destroyed by overheating when sealing off the glass tube for storage of the extract.

§ Extract may have been too dilute.

‖ Some hormone may have been lost during dissection.

type values themselves, it is probable that production of hormone is essentially the same in both cases; and therefore the same curve is drawn for both brown and wild type (Fig. 1).

In both series a few values seem to be either too high or too low as compared to the majority. Various reasons may be given as possible explanations for these aberrant color values. First, injury to the discs and prolonged exposure in Ringer's solution during dissection may account for some loss of hormone; second, although hormone extracts may be kept indefinitely when properly sealed off in glass tubes (Tatum,

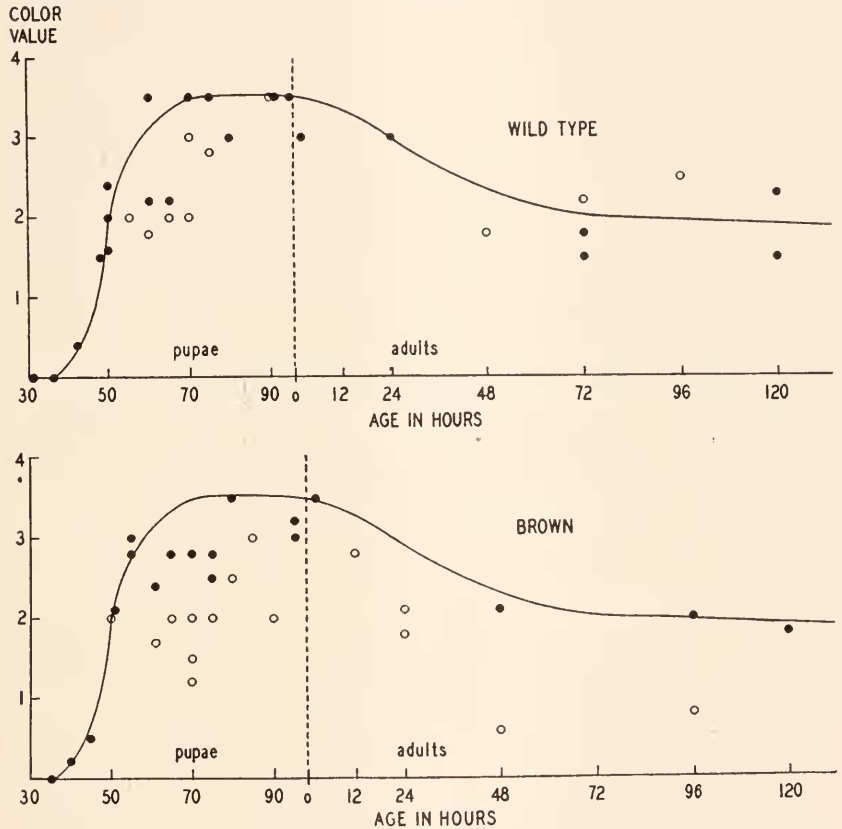

FIG. 1. Measurement of v^+ hormone in wild type and in brown eyes at various stages of development. (Experimental errors may reduce but not increase the apparent amount of hormone. Therefore the maximal values are considered more significant than the average, and the curves are plotted accordingly.)

unpublished), care must be taken to avoid overheating and evaporation, which may have occurred in some instances. A third source of error may be simply a statistical one. In some experiments the larval mortality was unusually high due possibly to bacterial infection. These cases, when only one fly emerged, may not be representative of a normal amount of injected hormone, since some variability in the amount in-

jected is inherent in the technique. Values believed to be unreliable for one or more of the above reasons are indicated in open circles in Fig. 1.

TABLE II

Amount of v^+ hormone present in eyes of wild type and brown flies.

Donors		No. of Hosts		Color Values		Units per donor*
Age in hours	Number	Injected	Emerged	Range	Mean	
Wild Type						
1–4	25	?	6	2.5–3.0	2.83	1.70
24	25	6	2	2.9–3.0	2.95	1.70
48	25	8	7	0.8–1.8	1.53	0.61§
72	25	7	5	1.0–1.5	1.34	0.47
72	25	9	6	0.5–1.8	1.33	0.61
72	25	4	2	1.8–2.2	2.00	0.88‡
96	25	4	2	2.0–2.5	2.25	1.13‖
120	25	8	5	1.5–2.3	1.82	0.94
120	25	8	5	0.8–1.5	1.02	0.47
144	25	5	3	1.8–2.0	1.87	0.72
Brown						
1–4	25	5	2	3.4–3.5	3.45	2.66
12	25	8	4	2.0–2.8	2.35	1.44‡
24	25	5	1	2.1	2.10	0.85†
24	25	7	3	1.0–1.8	1.43	0.61‡
48	25	6	2	2.0–2.1	2.05	0.85
48	25	9	2	0.4–0.6	0.50	0.21‡
96	25	5	2	2.0	2.00	0.72
96	25	6	3	0.4–0.8	0.57	0.25‡
120	25	6	4	1.5–1.8	1.70	0.61
120	25	6	5	1.7–1.8	1.76	0.61
144	25	6	2	1.0–1.5	1.25	0.47
144	25	6	3	0.0–0.5	0.23	0.19‡
168	25	6	2	0.7–1.3	1.00	0.40

* Values calculated from the maximal color modifications.

† Not reliable statistically.

‡ Some hormone may have been destroyed by overheating when sealing off the glass tube for storage of the extract.

§ Some hormone may have been lost during dissection.

‖ Extract may have been too concentrated.

The results for extracts of adult eyes (Table II and Fig. 1) indicate a significant decrease in the amount of hormone only during the first few days after emergence. The value obtained from wild type flies shortly after emergence seems to be low, but because the data were obtained from only a single extract, the value may not be significant.

It should be noted that data from single extracts are not quantitatively accurate, but the trend of the whole series is believed to be significant.

Extracts from Wild Type Eyes Grown in Vermilion Brown Hosts

Whether or not eye tissues themselves produce v^+ hormone was tested by transplanting optic discs from wild type larvae close to puparium formation into vermilion brown hosts of the same age, which are incapable of producing this hormone. Since it is known that the implants contain no hormone at the time of the operation (see above), its subsequent appearance would indicate production by the implant organ itself. After emergence of the hosts the implant eyes were recovered and extracted. The results of injection of these extracts show that the transplanted eyes had produced v^+ hormone (Table III).

TABLE III

Production of v^+ hormone by transplanted wild type eyes.

No. Eyes Recovered	No. of Hosts		Color Values		Units per* donor
	Injected	Emerged	Range	Mean	
50	7	5	0.8–2.2	1.55	0.88
50	7	1	2.0	2.00	0.72

* Values calculated from the maximal color modifications.

DISCUSSION

The results described above are in reasonable agreement with those of Tatum and Beadle (1938) in which dried whole pupae were extracted. For example, they recovered about 8 units of hormone per pupa, while the maximum amount obtained here was 3.36 units per pair of eyes. Other work has shown that the lymph (Beadle, Clancy, and Ephrussi, 1937), fat bodies and Malpighian tubes (Beadle, 1937) also contain the hormone. These sources may account for most of the difference in units noted.

Ephrussi (1938) discusses the general question of storage of insect hormones in organs and their utilization during specific developmental

stages, called "sensitive" or "effective periods." The evidence from these experiments bearing on this question indicates that v^+ hormone is not stored as such in eye tissues for any appreciable time before pigment appears, since detectable amounts of hormone appear at 40 hours and pigmentation at 47 hours after puparium formation, respectively.

Since the development of pigment in the eye is dependent upon the presence of the hormone, it is of interest to compare the time relations of hormone production and pigment formation. From the time of its appearance to about 55 to 60 hours after puparium formation pigmentation appears to be similar in wild type and brown. A light yellow color appears between 46 and 48 hours; this darkens to tan and finally to brown at about 60 hours. Referring to Fig. 1, it is evident that the amount of hormone increases greatly during this period, and at 60 hours, reaches a maximum which is maintained until emergence; possibly an equilibrium is reached with the eye using the hormone as rapidly as it is produced.

The experiments in which wild type optic discs were grown in vermilion brown hosts show that they produce only about one-third as much hormone as can be extracted from discs in their normal environment. This result is not inconsistent with the conclusion of Ephrussi and Chevais (1938) that a wild type eye grown in a vermilion host cannot itself produce enough hormone to attain full wild type pigmentation. On the other hand, if a wild type disc cannot produce enough hormone to complete its pigmentation (e.g. wild type in vermilion), the question arises as to why any hormone can be extracted from it. No explanation for this can be offered, but it is suggested that the utilization of hormone for pigment formation may depend upon a threshold concentration.

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Summary

Ringer's extracts of eye anlagen of wild type and brown stocks show that v^+ hormone appears in the optic discs approximately 40 hours after puparium formation (6 to 8 hours before onset of visible pigmentation).

The trend of the measurements of the amount of hormone in pupal discs during the course of development indicates that after its appearance the amount increases rapidly to a maximum which is probably maintained until emergence.

Adult eyes show a decrease in amount of hormone with increasing age.

Extracts of transplanted discs show that wild type eyes actually produce v^+ hormone, but in amounts insufficient for the development of full pigmentation.

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